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<p>(21) International Application Number: PCT/GB85/00183 (22) International Filing Date: 29 April 1985 (29.04.85)</p> <p>(31) Priority Application Number: 71683 (32) Priority Date: 27 April 1984 (27.04.84) (33) Priority Country: IL</p> <p>(71) Applicants (<i>for all designated States except US</i>): UNIVERSITY COLLEGE LONDON [GB/GB]; Gower Street, London WC1E 6BT (GB). YEDA RESEARCH AND DEVELOPMENT CO. LTD. [IL/IL]; P.O. Box 95, Rehovot 76100 (IL).</p> <p>(72) Inventors; and (75) Inventors/Applicants (<i>for US only</i>) : STANFORD, John, Lawson [GB/GB]; Mill House, Claygate, Marden, Kent (GB). COHEN, Irun, Robert [IL/IL]; 11 Hankin Street, Rehovot (IL). FRENKEL, Ayala [IL/IL]; 5 Hanasi Harishon Street, Rehovot (IL). HOLOSHITZ, Joseph [IL/IL]; 24 Hiber Street, Petach Tikva (IL). VAN EDEN, Willem [NL/NL]; Hooglandse Kerksteeg 3, N-2312 HR Leiden (NL). ROOK, Graham, Arthur, William [GB/GB]; 27 Glenloch Road, London NW3 4DJ (GB).</p>		<p>(74) Agents: COLLIER, Jeremy, Austin, Grey et al.; J. A. Kemp & Co., 14 South Square, Gray's Inn, London WC1R 5EU (GB).</p> <p>(81) Designated States: AT (European patent), AU, BE (European patent), CH (European patent), DE (European patent), FR (European patent), GB (European patent), IT (European patent), JP, LU (European patent), NL (European patent), SE (European patent), US.</p> <p>Published With international search report.</p>
<p>(54) Title: COMPOSITIONS FOR THE ALLEVATION, TREATMENT AND DIAGNOSIS OF ARTHRITIC DISEASE AND RELATED CONDITIONS</p> <p>(57) Abstract</p> <p>The invention provides pharmaceutical compositions for the alleviation of arthritic diseases. These contain as active ingredient mycobacteria or fractions of these, e.g. obtained by fractionation in certain solvents. The compositions can also be used for vaccinations. There is also provided an assay for the diagnosis of arthritic diseases, and a kit for carrying such an assay.</p>		

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COMPOSITIONS FOR THE ALLEVIATION, TREATMENT AND DIAGNOSIS
OF ARTHRITIC DISEASE AND RELATED CONDITIONS

The invention relates to preparations for preventing various arthritic afflictions, for alleviating symptoms of arthritic diseases and of other autoimmune diseases, and for their diagnosis.

5 The novel preparations are based on certain mycobacteria or on certain fractions obtained from mycobacteria. There were also developed certain clones of T-lymphocytes which can be used for diagnostic and therapeutic purposes.

Background of the Invention:

10 Millions of persons are afflicted with chronic forms of arthritis which are thought to involve autoimmunity to constituents of the joints or connecting tissues of the body. These conditions include rheumatoid arthritis, ankylosing spondylitis, Reiter's syndrome and other forms of 15 reactive arthritis. The etiology of these diseases is not known, but previous infection with various microbes seems to act as an inciting circumstance in genetically susceptible individuals. For example, patients with rheumatoid arthritis may show unusual immune reactivity to mycobacterial antigens 20 and immunization with the BCG strain of mycobacteria was found to lead to arthritis in 15 of 150 individuals. Ankylosing spondylitis has been associated with infection by Klebsiella or Yersinia species of bacteria and other cases 25 of arthritis by Salmonella, Snigella, etc. There is no evidence of active infection of joints by these microbes in the vast majority of cases and it has been postulated that microbial infection may trigger an aberrant, autoimmune response of the individual against his own antigens present in the joints.

30 Adjuvant arthritis (AA) is an experimental model of arthritis inducible by immunizing susceptible strains of rats to Mycobacteria. The disease which develops about 12 days after immunization has many of

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the features of rheumatoid arthritis and AA has been considered to be a model of rheumatoio arthritis.

Summary of the Invention

There are provided pharmaceutical preparations for the diagnosis, for the vaccination against, and for the treatment of various 5 autoimmune diseases and especially of arthritic conditons. There exists a family of chronic arthritic conditions such as rheumatoid arthritis, ankylosing spondylitis or Reiter's syndrome which are thought to arise from autoimmune processes in which the joints and other tissues are damaged by the immune system of the patient.

10 The triggering factors are unknown but it is believed that the infection with certain microbial agents may be important.

Adjuvant arthritis is considered to be an experimental model of autoimmune arthritis inducible in strains of rats by immunizing them to mycobacterial antigens.

15 According to the present invention there are provided pharmaceutical preparations based on certain mycobacteria and on fractions derived from such mycobacteria.

We have found that various types of mycobacteria, such as Mycobacteria H-37 RA, M. kansasii, M. vaccae, and similar strains may 20 be used as such or fractionated by the use of certain solvents to give a precipitate and a water soluble fraction, which latter is suitable for various vaccinations and curative purposes.

Mycobacteria H-37 can be fractionated by the use of an aqueous solution of acetone (66% acetone in water). There is obtained a 25 precipitate (AP) fraction and an acetone soluble (AS) fraction.

The immune response to the AS fraction leads to resistance to adjuvant arthritis; clones of lymphocytes that respond to AS, upon

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inoculation into naive rats, protect these against subsequent induction of adjuvant arthritis. Inoculation of such clones of lymphocytes into rats suffering from adjuvant arthritis markedly hastens their recovery from the arthritis.

5 It has been found that clones of T-lymphocytes which cause adjuvant arthritis respond (proliferate) to the AP fraction, but not to the AS fraction.

The SP and the AS fractions are immunologically cross-reactive with proteoglycans of normal joint cartilage, and therefore adjuvant

10 arthritis can be explained as a noxious autoimmune response to AP cross-reactive antigens of proteoglycans. Protection against adjuvant arthritis can be associated with a protective, or disease suppressive response to the AS cross-reactive antigens of proteoglycans.

Diagnostic tests for autoimmune arthritis and similar autoimmune
15 diseases can be based on the different immune reactivity of the tested persons to be AS and the AP fractions of mycobacteria and other bacteria associated with arthritis, or to the AS and AP cross-reactive antigens of proteoglycans. Immunization of patients to AS fractions of mycobacteria and other bacteria associated with arthritic conditions,
20 or to AS cross-reactive antigens or proteoglycans can be used for the prevention of autoimmune arthritis and for the treatment of arthritic diseases.

It has been discovered that certain lines and clones of T-lymphocytes selected for their reactivity to mycobacteria can be used
25 for producing arthritis upon inoculation into irradiated rats.

One line, designated as A2 was found to induce arthritis upon intravenous injection into irradiated rats. The same line, A2 is effective in vaccinating unirradiated rats against subsequent AA induced by active immunization to Mycobacteria.

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Cell line A2 has been cloned and there were obtained two distinct clones, designated as A2b and A2c, respectively. A2b causes arthritis but does not vaccinate against it; clone A2c does not cause arthritis but vaccinates against it (see Table 1).

5 In addition to preventing arthritis, clone A2 can be used to treat AA. Figure 1 shows the result of an experiment in which rats suffering from AA were inoculated twice (on days 16 and 17 after the induction of disease) with clone A2c, or with a control, irrelevant clone of T-lymphocyte. The rats inoculated with clone A2c went into rapid
10 remission. Six months later the A2c treated rats had normal joints while the control rats had ankylosis and deformities of the paws.

Thus, clones A2b and A2c can be used to identify antigens associated with arthritogenicity or with suppression of arthritogenicity. Both clones respond to whole mycobacteria.

15 Clone A2b responds to the AP fraction but not to the AS fraction defined above; protective clone A2c responds to AS and only slightly to AP. Both A2b and A2c respond to cartilage proteoglycan, see Table 2.

Results presented in Table 3 demonstrate that anti-AP and anti-AS
20 antisera recognize cross-reactive antigens in cartilage. AP and AS induce different classes of antibodies, IgG and IgM, respectively, which indicates that these fractions induce functionally different responses. IgG is associated with AP and arthritogenicity while IgM is associated with AS and suppression of arthritogenicity.

25 Rats were immunized with Mycobacteria, with water, and with AS in oil, see Table 4. The mycobacteria inoculated rats developed AA as expected, while the water and AS inoculated rats did not. After 35 days the rats were challenged with mycobacteria in oil to induce active

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AA. Rats that had suffered primary AA were resistant to a second bout; those inoculated with water only were susceptible to AA, whereas the AS inoculated rats were totally resistant to AA. Thus, the As fraction is arthritis suppressive, while not being arthritogenic. Moreover, AS can be used to activate cells of the A2 line to provide treatment of AA after its onset. (Figure 2). The effective dosage varies, it is generally about 1 to 20 mg/kg, preferably about 2 to 10 mg/kg. This demonstrates that when AA was induced in rats by Mycobacteria, after 16 days, when arthritis had developed, some of the rats were inoculated intravenously with line A2 that had been activated with Mycobacteria or with the As fraction. The Mycobacteria treated A2 cells arrested the arthritis, while the AS treated A2 cells induced a full remission of the disease.

Table 5 demonstrates the proliferative responses of peripheral blood mononuclear cells of rheumatoid arthritis (RA) patients and controls, to mycobacterial antigens.

Several different species of mycobacteria were tested 20 as to whether clones A2b and A2c recognized their differences. One of these, mycobacteria, M. vaccae was recognized in an in vitro proliferation test by the protective clone A2c but not by the arthritogenic clone A2b (Table 6). This finding indicated that M. vaccae was relatively rich in protective 25 antigens and poor in arthritogenic antigens. Accordingly tests were carried out to evaluate the effect of M. vaccae on adjuvant arthritis (Table 7). The strain of M. vaccae used was that deposited at the National Collection of Type Cultures (NCTC) Central Public Health Laboratory, Colindale 30 Avenue, London NW9 5HT on February 13th 1984 under the number NCTC 11659. In the first experiments, rats were inoculated with M. vaccae and then a week later adjuvant arthritis was induced by immunizing the rats with M. tuberculosis. It was found that prior inoculation with M. 35 vaccae prevented the development of arthritis. Thus M. vaccae is

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effective as a prophylactic vaccine against adjuvant arthritis.

In the second series of experiments, adjuvant arthritis was first induced by immunization with *M. tuberculosis* and 3 weeks later, when all the rats were suffering from arthritis, some rats were inoculated 5 with *M. vaccae* in oil, or with oil alone. Those receiving *M. vaccae* in oil had a remission in arthritis in several days while the control group of rats continued to suffer from severe arthritis (Figure 3).

We have evidence that rheumatoid arthritis patients have T-lymphocytes that respond to the arthritogenic fraction of *M. tuberculosis* (Table 5), indicating that similar immunologic processes may occur in both adjuvant arthritis in rheumatoid arthritis.

According to the invention there are also provided compositions for the diagnosis of arthritic diseases and an assay for this purpose, based on the use of whole mycobacteria or on the use of certain 15 fractions thereof, obtained by the separation of mycobacteria. Such separation can be effected in a suitable solvent system, whereby there is obtained a soluble fraction and an insoluble one (precipitate). Each of these can be further fractionated and purified, until specifically active substances are obtained. Such fractions can be 20 used for various types of assays for the above purpose, such as:

- a 1. a lymphocyte proliferation test, or determination of any entity indicative of such proliferation;
- a 2. indicative of the measure of lymphocyte activation are also changes which can be assayed by standard means so as to establish 25 the presence and degree of lymphocyte activation: amongst these there may be mentioned:
 - a. production of lymphokines (such as interleukin-2 (IL-2);
 - b. gamma interferon;

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- c. migration inhibition factor (MIF);
 - d. expression of membrane markers, such as IL-2 receptor; peanut agglutination receptor.
 - e. expression of enzymes such as heparanase;
- b. determination of antibody titer in absolute terms or as a ratio of the values obtained by different fractions, said values or ratios being indicative of the presence or absence of the disease. Quantitative values obtained are of use in establishing the severity of the disease.

For carrying out such assays, there can be provided means in kit form, comprising one or more of the above defined fractions with suitable adjuvants and auxiliary components. Standardized kits with reference and calibration means are of value in the rapid and convenient determination of arthritic disease and its stage and/or severity.

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Legend to the figures:

Figure 1: Treatment of Adjuvant Arthritis (AA) Using Clone A2c:

AA was induced in Lewis strain rats by inoculation of MT. Sixteen and seventeen days later groups of 10 rats each were injected intraperitoneally with 2×10^7 cells of clone A2c (open circles) or central clone Cla (closed circles). The 5 rats were observed for the severity of arthritis on a clinical scale of 1 to 16. Upon examination 6 months later, recipients of clone A2c were free of disease while recipients of Cla had severe ankylosis of the paws.

Figure 2: Treatment of Adjuvant Arthritis by Injection of A2 Line Cells:

Adjuvant arthritis was induced in 30 Lewis rats by active 10 immunization with complete Freund's adjuvant on day 0. On day 16, after arthritis had developed in all rats, they were divided into three groups. A control group of 10 rats (solid circles) was not treated. A second group of 10 rats was treated by a single intravenous inoculation of 2×10^7 15 A2 line cells that had been activated using whole myco-bacterial organisms (o). The third group of 10 rats was inoculated with A2 line cells that had been activated with the AS fraction (triangles). Severity of arthritis was assessed on a clinical scale of 1 to 16.

Figure 3: Treatment of Adjuvant Arthritis by Injection of M. vaccae Cells

20 AA was induced in Lewis strain rats by immunization with M. tuberculosis and 2 and 4 weeks later, when all the rats were suffering from arthritis, some rats were inoculated with M. vaccae (1 mg.) in oil (incomplete Freund's adjuvant) (open circles) or with the oil alone (solid circles). Severity 25 of arthritis was assessed on a clinical scale of 1 to 16.

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TABLE 1: Production and/or prevention of adjuvant arthritis (AA) by

T-lymphocytes transferred (2x10 ⁷)	T-lymphocyte line A2 and clones A2b and A2c		Line-mediated AA induced by MT 35 days after line transfer		
	Recipient rats	arthritis (750R)	% Incidence (no. rats)	Mean day of onset	Duration (days)
A2	No	No	89 (76)	12.9	56
	Yes	No	81 (42)	13.6	55
A2b	No	No	0 (69)	-	-
	Yes	Yes	0 (38)	-	-
A2c	No	No	91 (22)	13.7	53
	Yes	Yes	93 (14)	13.9	54
	No	No	0 (15)	-	-
	Yes	No	0 (15)	-	-

Irradiated (750R) or non-irradiated Lewis rats were injected intravenously with 2x10⁷ cells of line A2 or cloned sublines A2b or A2c-10. 35 days later active AA was induced by an intradermal injection of killed *Mycobacterium tuberculosis* organisms in oil (MT). Control groups consisting of irradiated or non-irradiated rats were injected with MT 35 days after irradiation.

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TABLE 2: Responses of clones A2b and A2c to antigens

Clone	In vivo effect	Response to antigens			
		Whole mycobacteria	AP	AS	Cartilage proteoglycan
A2b	arthritogenic	+	+	-	+
A2c	protective, therapeutic	+	+	+	+

TABLE 3: Rabbit antibodies to AP or AS recognize joint cartilage

Rabbit antiserum	Immunofluorescent staining of joint cartilage	Inhibition of staining with proteoglycan
normal	none	-
anti-AP	IgG	Yes
anti-AS	IgM	Yes

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TABLE 4: Active Immunization to AS Protects Rats against AA

Primary Immunization	Inoculum in oil 1 mg	% incidence of arthritis	Secondary challenge with mycobacteria in oil (MT)	% incidence of arthritis
Mycobacteria		100	0	0
Water		0	100	0
AS		0	0	0

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TABLE 5: Proliferative responses of peripheral blood mononuclear cells of rheumatoid arthritis (RA) patients and controls to mycobacterial antigens

<u>Group</u>	<u>No.</u>	<u>Mean stimulation index</u>			<u>Significant response to*</u>	<u>Ratio of responses</u>
		<u>PPD</u>	<u>AP</u>	<u>AS</u>		
<u>patients</u>						
RA	17	18.4	13.6	1.2	14/17	0/17
Osteoarthritis	12	15.0	5.6	7.0	7/12	8/12
Normal	8	13.2	3.9	4.9	4/8	5/8
<u>controls</u>						

*Stimulation index > 2.0

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TABLE 6: Therapeutic clone A2c recognizes M. vaccae, arthritogenic clone A2d does not.

Clone	In vitro proliferation response (H^3 -thymidine incorporation, cpm $\times 10^{-3}$)		
	No antigen	<u>M. tuberculosis</u>	<u>M. vaccae</u>
A2b	2 ₋₁	6 ₋₅	4 ₋₁
A2c	1 ₋₁	62 ₋₆	60 ₋₉

Clones A2b and A2c were assayed for their in vitro proliferative responses to M. tuberculosis or M. vaccae organisms in a standard test (2.5×10^4 clone cells, 2×10^6 irradiated accessory cells and 2 ug of mycobacteria extract per well, H^3 -thymidine incorporation for 18 h, after 24 h of incubation).

Results are expressed as cpm.

TABLE 7: Treatment with M. vaccae induces resistance to adjuvant arthritis.

Treatment	Arthritis induced by <u>M. tuberculosis</u> 3 months later	
	Incidence	Clinical grade
Oil (control)	100%	Severe
<u>M. vaccae</u> in oil	0%	0

Groups of 13 Lewis rats were treated by intracutaneous inoculation of M. vaccae (1 mg) in oil (incomplete Freund's adjuvant) or by oil alone (control). Three months later, susceptibility to induction of adjuvant arthritis was tested by inoculating the rats with M. tuberculosis (1 mg) in oil.

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CLAIMS

1. A composition for alleviation of the symptoms of and for the treatment or diagnosis of arthritic diseases which comprises, as active ingredient, a mycobacterium or a fraction or secretion thereof.

5 2. A composition according to claim 1, wherein the mycobacterium is M. vaccae.

3. A composition according to claim 1 or 2 comprising a portion obtained by fractionation of such mycobacteria in a suitable solvent system, and separating 10 therefrom a soluble fraction used as active component, or a secretion of such mycobacteria into a culture medium.

4. A composition according to claim 3, wherein the solvent system for fractionation is an acetone/water mixture.

15 5. A composition according to claim 4, wherein the solvent system used for fractionation is an acetone/water mixture of about 2/1 by volume.

6. A composition according to claim 2, wherein the active ingredient is the whole organism of M. vaccae.

20 7. A composition according to any of claims 1 to 6, in the form of an injectable preparation, as oral dosage form, or as dosage form applicable to any body cavity.

8. A composition according to any of claims 1 to 7, wherein the active ingredient is present together with 25 an adjuvant.

9. A composition according to any of claims 1 to 8, wherein the active ingredient is in unit dosage form, containing from about 2 mg to about 10 mg/kg weight of the patient.

30 10. A composition according to any of claims 1 to 9, for use in therapy for the immunization against, and for the treatment of, arthritis.

11. A composition according to any of claims 1 to 9, for use in therapy for the diagnosis of arthritic diseases 35 by determination of lymphocyt proliferation; determination

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of any entity indicative of such proliferation; or determination of antibody titre.

12. A composition according to claim 11, comprising a soluble and/or non-soluble fraction of a mycobacterium,
5 which may be further separated or purified.

13. A composition according to claim 11 or 12, wherein the determination is effected by measuring absolute values or the ratio of values obtained by the use of different fractions.

10 14. A composition according to claim 11, 12 or 13, for the determination of the presence or absence of an arthritic disease, and/or its severity, in kit form, comprising such fraction or fractions with required adjuvants and auxiliaries and with calibration means.

15 15. A method for the alleviation or treatment of arthritic disease or a related condition which comprises administering to a patient suffering therefrom or subject thereto an effective amount of a composition as claimed in claim 1.

20 16. A method of the diagnosis of arthritic disease or a related condition which comprises determining lymphocyte proliferation or any entity indicative of each proliferation or determining antibody titre, using in such determination a composition as claimed in claim 1.

25 17. A method as claimed in claim 15 or 16, wherein the composition is as claimed in any one of claims 2 to 14.

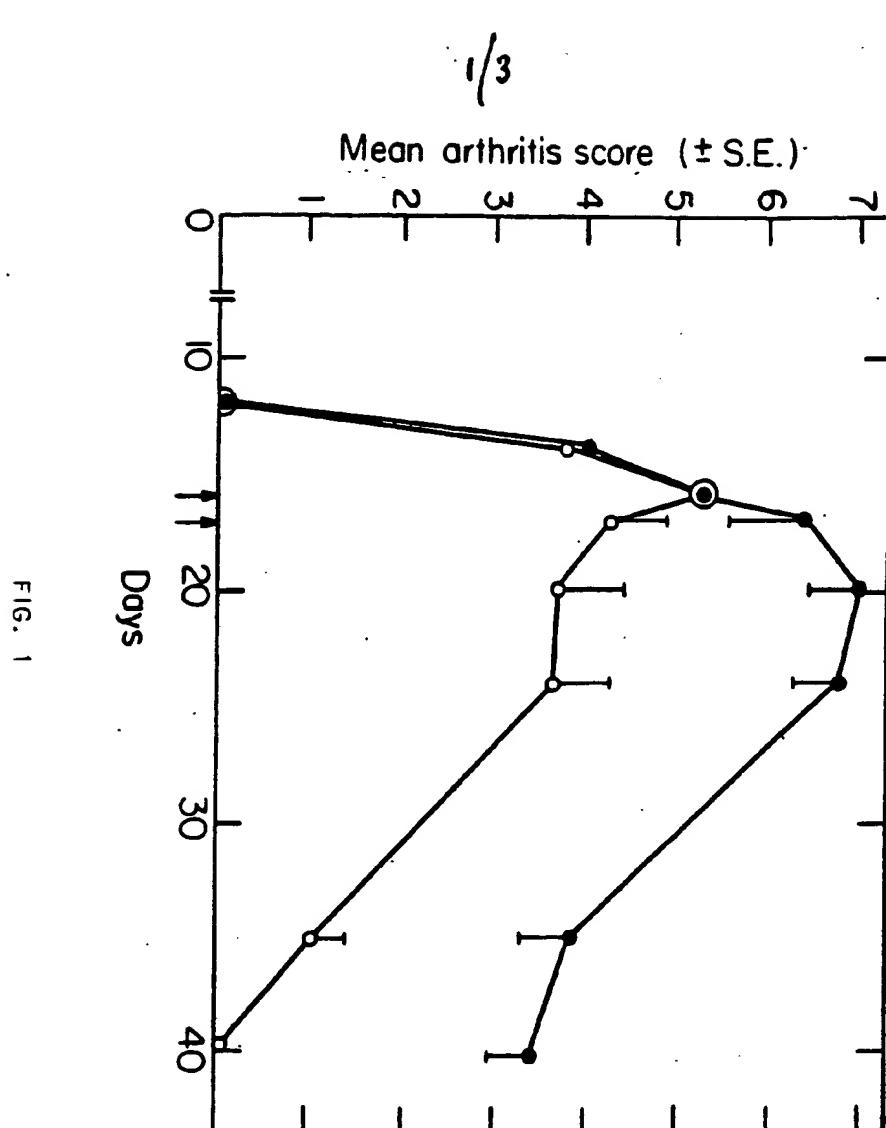


FIG. 1

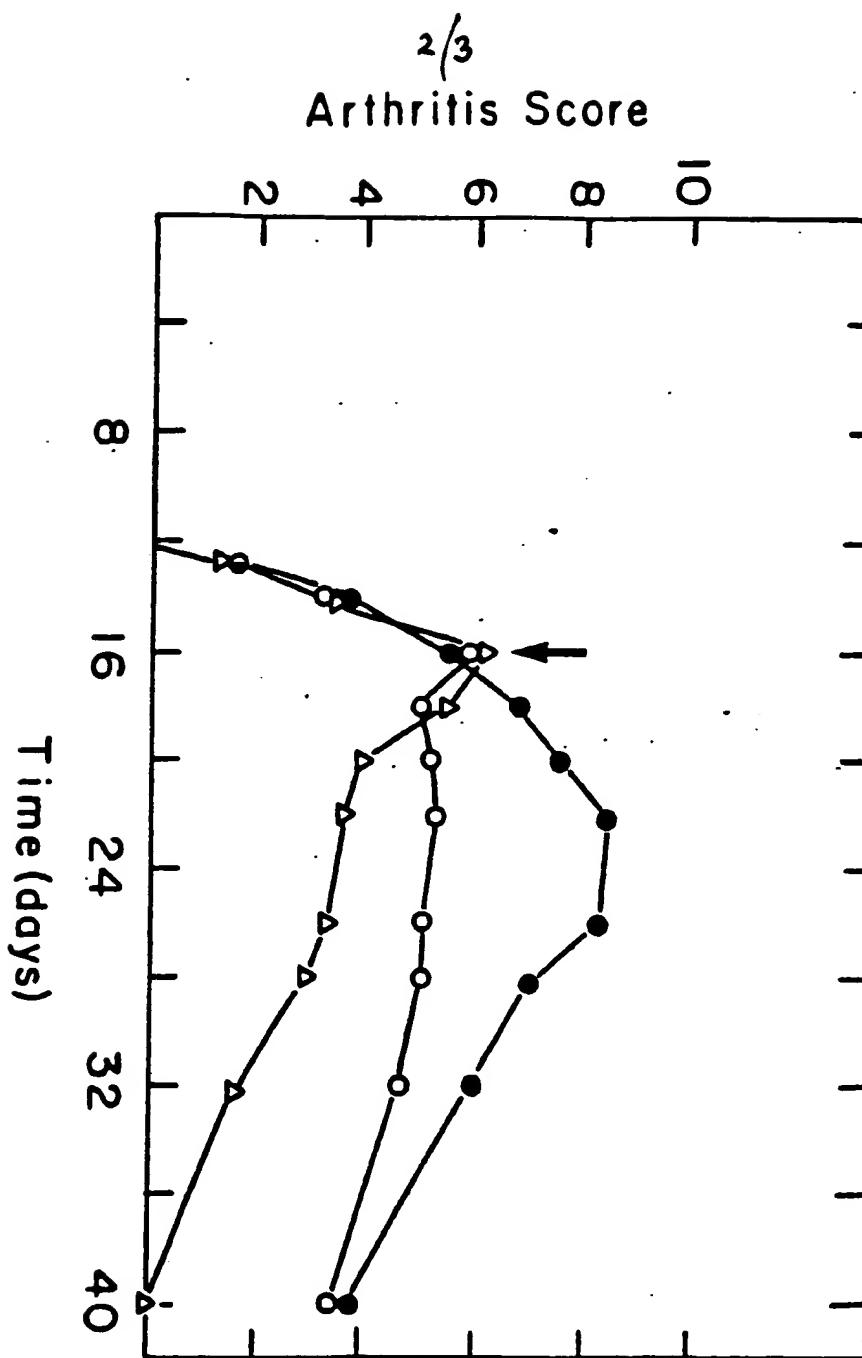


FIG. 2

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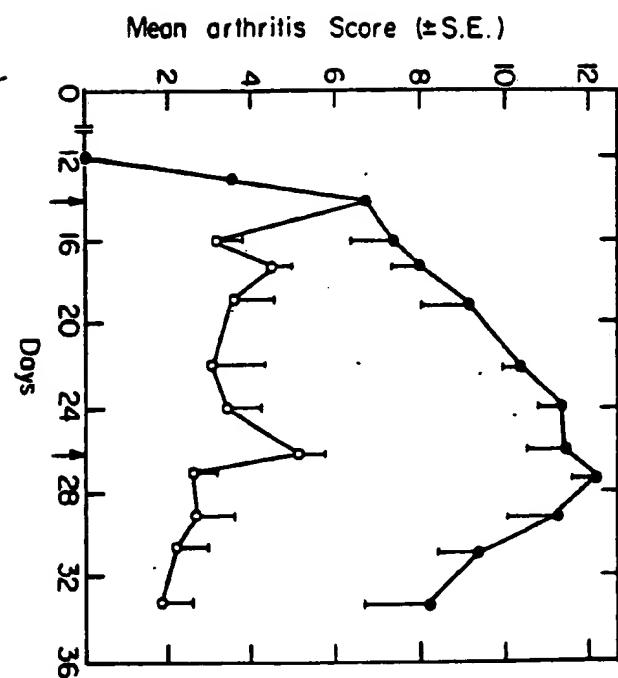


FIG. 3

INTERNATIONAL SEARCH REPORT

International Application No PCT/GB 85/00183

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) ⁴

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC ⁴: A 61 K 35/74; A 61 K 39/04

II. FIELDS SEARCHED

Minimum Documentation Searched ⁷

Classification System	Classification Symbols
IPC ⁴	A 61 K

Documentation Searched other than Minimum Documentation
to the Extent that such Documents are Included in the Fields Searched ⁸

III. DOCUMENTS CONSIDERED TO BE RELEVANT⁹

Category ¹⁰	Citation of Document, ¹¹ with indication, where appropriate, of the relevant passages ¹²	Relevant to Claim No. ¹³
X	EP, A, 0045237 (BERRI BALZAC) 3 February 1982, see claims 1,2,4; page 9, lines 25-26; page 12, lines 14,17	1,7,10-13, 17
X	Chemical Abstracts, volume 68, nr. 25, 17 June 1968, (Columbus, Ohio, US) P. Jolles et al.: "Wax D, peptidoglycolipid of Mycobacterium tuberculosis: further purification and study of an adjuvant arthritis-inhibiting subfraction", see page 10822, abstract nr. 112335Y, Immunology 14(2), 159-63(1968)	1,7,10-13, 17
A	Biological Abstracts, volume 73, nr. 11, 1982, (Philadelphia, Pa, US) G.M. Bahr et al.: "Inhibition of the proliferative response of peripheral blood lymphocytes to mycobacterial or fungal antigens by co-stimulation with antigens from various mycobacterial species", see page 7931-2, abstract nr. 76033, Immunology, 44(3); 593-598, 1981	1-14, 16, 17

* Special categories of cited documents: ¹⁰

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the International filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the International filing date but later than the priority date claimed

"T" later document published after the International filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"Z" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search
6th August 1985

Date of Mailing of this International Search Report

29 AOUT 1985

International Searching Authority

EUROPEAN PATENT OFFICE

Signature of Authorized Officer

G.L.M. Kruydenberg

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category*	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
A	Biological Abstracts, volume 69, nr. 1, 1980, (Philadelphia, PA, US) S.R. Watson et al.: "Delayed hypersensitivity responses in mice and guinea pigs to Mycobacterium leprae, Mycobacterium vaccae and Mycobacterium nonchromogenicum cytoplasmic proteins", see page 306, abstract nr. 2847, Infect. Immun., 25(1), 229-236, 1979 --	1-14, 16, 17
A	Infection and Immunity, volume 20, nr. 2, May 1978, (Washington, US) F.M. Collins et al.: "Immune response to persistent Mycobacterial Infection in Mice", see pages 430-438, page 437, lines 30-54 --	1-14, 16, 17
A	FR, A, 2184531 (A.N.V.A.R.) 28 December 1973, see claims 1, 5 and 20 --	1-14, 16, 17
A	FR, A, 2275224 (A.N.V.A.R.) 16 January 1976, see claims 1, 7, 12 and 21 -----	1-14, 16, 17

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET**V. OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE:**

This International search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. Claim numbers 15, 17 because they relate to subject matter not required to be searched by this Authority, namely:

Methods for treatment of the human or animal body by surgery or therapy, as well as diagnostic methods. PCT Rule 39.1(iv)
 °°) claim 17 partially not searchable

2. Claim numbers, because they relate to parts of the International application that do not comply with the prescribed requirements to such an extent that no meaningful International search can be carried out, specifically:

3. Claim numbers....., because they are dependent claims and are not drafted in accordance with the second and third sentences of PCT Rule 5.4(a).

VI. OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING:

This International Searching Authority found multiple inventions in this International application as follows:

1. As all required additional search fees were timely paid by the applicant, this International search report covers all searchable claims of the International application.

2. As only some of the required additional search fees were timely paid by the applicant, this International search report covers only those claims of the International application for which fees were paid, specifically claims:

3. No required additional search fees were timely paid by the applicant. Consequently, this International search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

- The additional search fees were accompanied by applicant's protest.
 No protest accompanied the payment of additional search fees.

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON

INTERNATIONAL APPLICATION NO. PCT/GB 85/00183 (SA 9446)

This Annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 19/08/85

The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A- 0045237	03/02/82	FR-A, B 2487198 JP-A- 57140792 US-A- 4404194 AT-B- E8146	29/01/82 31/08/82 13/09/83 15/07/84
FR-A- 2184531	28/12/73	NL-A- 7306964 DE-A- 2325299 GB-A- 1438556 CA-A- 1003771 JP-A- 50004228	21/11/73 06/12/73 09/06/76 18/01/77 17/01/75
FR-A- 2275224	16/01/76	NL-A- 7507376 BE-A- 830486 DE-A- 2527636 GB-A- 1516507 JP-A- 51032794	23/12/75 22/12/75 08/01/76 05/07/78 19/03/76